A new spin on lymphocyte homing

New evidence confirms the multi-step model for leukocyte migration through endothelia, but with a twist. The selectins are not always required, and some lymphocyte subsets rely heavily on integrins for their migration to tissues.

The migration of leukocytes through the body facilitates immune surveillance as well as directing immune responses to antigen-challenged tissues. Functionally distinct subsets of leukocytes are required at different stages during an inflammatory response, or in different types of tissue in response to particular types of antigen. The migration of these subsets is regulated by a variety of adhesion molecules and chemotactic cytokine receptors, and by the constitutive or induced expression of adhesion ligands on the endothelia that the leukocytes bind to and traverse.

The three-step model

Leukocyte binding to endothelia in inflamed tissues was long viewed in a simplistic way. An adhesion molecule on leukocytes was thought to interact with a ligand on the relevant endothelium. The process turns out to be much more complex than that. Observations of leukocyte binding to endothelia *in vivo*, using intravital microscopy, or *in vitro*, using procedures that simulate blood flow, show leukocyte–endothelium binding and the exit of leukocytes from the bloodstream proceeds through a characteristic series of steps.

The initial contact between leukocyte and endothelium — tethering — is followed by 'rolling' of the leukocyte along the vessel wall. This rolling is transient and reversible, although some leukocytes stop, establish a firm adhesion, flatten, and then migrate through the vessel wall. When these physiological observations of leukocyte behavior were combined with a new understanding of the properties of different adhesion molecules, a new paradigm emerged for how leukocytes bind to endothelium [1–3]. This 'three-step model' assumes that multiple adhesion receptor–ligand pairs act in a sequential and overlapping manner. The binding of a cell to a particular endothelium is determined by a combination of factors, rather than by a single adhesion event.

Leukocytes in the blood pass through venules at high shear rates, so special adhesion mechanisms are needed to slow them, to allow them to adhere and de-adhere in the rolling process. Studies with neutrophils implicated the selectin family of adhesion molecules in the initial contact and rolling process [4]. Selectins have fast 'on' and 'off' rates for binding to their ligands, and they bind with high tensile strengths [5], making them ideal molecules for mediating initial attachment and rolling. The selectin family comprises P-selectin, expressed by platelets and inflamed endothelium, E-selectin, expressed on inflamed endothelium, and L-selectin, expressed on most leukocytes. Sialylated ligands for E- and P-selectins are also expressed on subsets of lymphocytes, whereas ligands for lymphocyte L-selectin are expressed on high endothelial venules (HEVs), the specialized venules of lymph nodes. L-selectin plays an important role in the homing of lymphocytes to peripheral lymph nodes [6] — particularly the homing of unactivated/naive T cells [7,8] — whereas the E- or P-selectin on inflamed endothelium interacts with ligands on lymphocytes to mediate contact and rolling [9].

The second step in the three-step model is an activation event that causes cell adhesion molecules of the integrin family to change their conformation and binding affinity for ligands. Chemokines, of which there are 20 or more, bind to G-protein-coupled receptors on leukocytes [10], and at present are considered to be the most likely signals to lead to integrin activation [1,11]. The third step occurs when a cell migrates through the endothelium to the tissue. This process is particularly dependent on interactions between LFA-1, an integrin, and its ligand, ICAM1, and probably also on CD31, an adhesion molecule of the immunoglobulin superfamily [12].

An important aspect of the three-step model is that it provides flexibility for the control of leukocyte migration [1]. Tethering can be provided by one of three selectins, the activation of integrins by one of numerous chemokines or receptors, and firm adhesion by one of five or more integrins. The number of possible combinations of molecular interactions for tethering, activation, and arrest is therefore enormous [1–3], providing an intricate control mechanism that allows subsets of cells to enter particular tissues at specific times during an inflammatory response.

How lymphocytes home

Endothelial cells are the regulators of lymphocyte traffic [3,13,14]. There are two fundamentally different types of endothelium that lymphocytes bind to and cross: the specialized HEVs of organized lymphoid tissue, such as lymph nodes and the gut-associated Peyer's patches, and the flatter endothelia of normal and acutely inflamed tissues. The migration of lymphocytes across HEVs is substantial — approximately one in every four lymphocytes is extracted from the blood. Migration across flat endothelia, by contrast, is minimal, except in cases of inflammation. Different HEVs and various endothelia throughout the body extract different subsets of lymphocytes.

These differences operate at two levels. First, the subset of lymphocytes that binds to, and crosses, HEVs is usually

qualitatively different from the subset that crosses flat endothelium [15]. Second, the HEVs of mucosal tissues are different from those of peripheral lymph nodes [13], and the flat or inflamed endothelium of the skin is different from that of the lamina propria lining the gut. For instance, vascular addressins and other endothelial adhesion ligands are often expressed in a highly tissue-specific manner [14], resulting in functionally distinct lymphocyte migration pathways. Gowans showed many years ago [16] that rat lymphoblasts isolated from the gut migrate preferentially back to the gut. Studies in mice and sheep likewise demonstrated the existence of a peripheral-homing and a mucosal-homing subset of lymphocytes [14,17]. The purpose of having these distinct pools may relate to economy and rationalization: it makes sense that cells stimulated by antigen in the gut should preferentially re-colonize regions of the gut in the likelihood that the relevant antigen will be found there. Alternatively, cells may need to be directed to mucosal surfaces in order to promote particular functions, such as producing the cytokines characteristic of Th_2 T cells, or the production of immunoglobulin A, both of which are important for mucosal immunity.

Refining the three-step model

Although originally proposed in general molecular terms [1], it has subsequently been widely assumed that the tethering and arrest phases of leukocyte–endothelial cell interactions are the exclusive provinces of selectins and integrins, respectively. However, recent studies show that selectins are not the only molecules capable of mediating the initial attachment and rolling of lymphocytes [18–22].

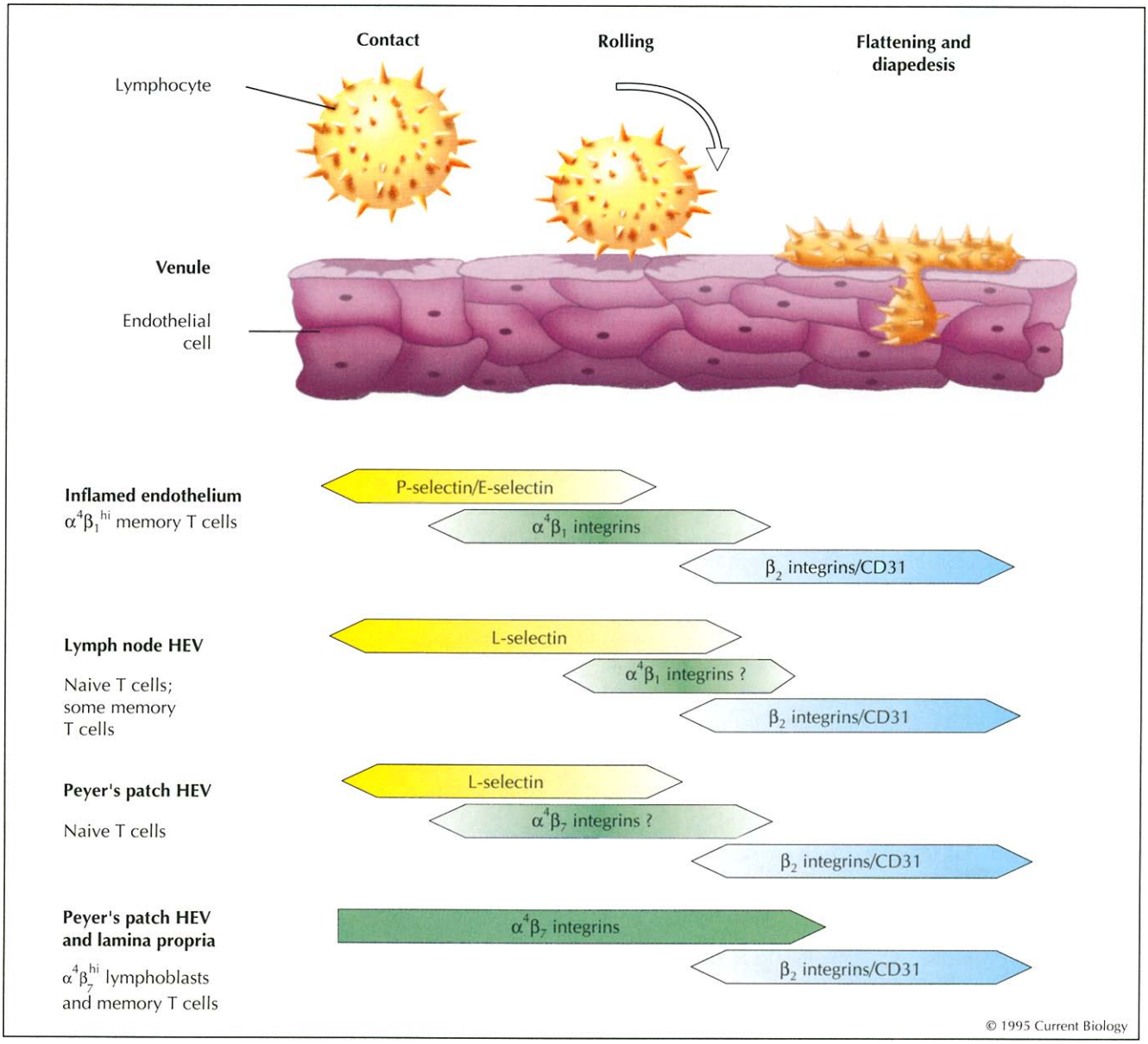

Fig. 1. The overlapping roles of selectins, α^4 integrins, and β_2 integrins/CD31 in lymphocyte tethering, rolling, and diapedesis. The relative involvement of these three classes of adhesion molecules varies between different endothelia and various populations of lymphocytes. In particular, the role of selectins in tethering can be performed by α^4 integrins. Adapted from Bargatze *et al.* [19].

This is an important consideration for lymphocyte homing, because it suggests that, under some circumstances, certain molecules may play a predominant role in determining the migration pathway of particular cells. Butcher's group [18] showed that, under flow conditions, the $\alpha^4\beta_7$ integrin on T cells or lymphoma cell lines could mediate the initial attachment and rolling on MAdCAM-1 (the mucosal vascular addressin expressed on Peyer's patch HEVs and lamina propria endothelium). In addition, a number of studies show that $\alpha^4\beta_1$ integrin on the surface of transfected cells or lymphocytes participates in their rolling [21], and even tethering in shear flow, on cells bearing the ligand VCAM-1 [18,20,22]. Tethering mediated by α^4-subunit-containing integrins does not require pre-existing integrin activation, although it is enhanced when integrins are activated, and may be important when VCAM-1 or MAdCAM-1 is present on endothelial cells at only low densities.

Scanning electron microscopy has revealed an interesting feature of leukocytes — the presence on their surfaces of numerous microvilli. These microvilli seem to be important for the contact of cells to endothelium under flow conditions. L-selectin is displayed selectively on the microvilli, whereas integrins containing a β_2 subunit — for example, LFA-1, the $\alpha^L\beta_2$ integrin — are excluded from the microvilli [18,23,24]. A model is emerging whereby adhesion molecules on the tips of microvilli brush endothelial cells as the lymphocyte contacts and rolls along the vessel wall. Adhesion molecules on other parts of the lymphocyte cell membrane then function in subsequent adhesion processes, such as those occurring when the lymphocyte is flattened and migrates through the endothelium.

What then is the role of α^4-containing integrins? Berlin *et. al.* [18] show a dramatic localization of $\alpha^4\beta_7$ integrin to the tips of microvilli — similar to the localization of selectins — supporting the notion that this integrin can function in tethering and the rolling. It seems probable that the adhesion molecules on the microvilli slow a cell down, and facilitate rolling, whereas adhesion molecules associated with other parts of the cell membrane may have closer interactions with cytoskeletal elements or signal transduction molecules necessary for cell movement. Thus, the function of adhesion molecule is regulated not only through their expression, or conformational changes, but also by their topographical distribution within the cell membrane.

How does the expression and function of selectins and integrins determine homing behavior *in vivo*? Bargatze *et al.* [19] have addressed this question by examining the interactions of various populations of lymphocytes with endothelial cells of mouse Peyer's patches using video microscopy and combinations of function-blocking monoclonal antibodies. L-selectin (the selectin of lymphocytes) was shown to be the most important molecule for the initial tethering of naive lymphocytes to Peyer's patch HEVs, in keeping with previous *in vivo* data [25,26]. These experiments also showed that a cell cannot bypass the $\alpha^4\beta_7$-mediated initial tethering and rolling step. Rolling mediated by L-selectin alone did not permit efficient engagement of LFA-1 with its ligand. Antibodies to $\alpha^4\beta_7$ slowed rolling velocity, with $\alpha^4\beta_7$ apparently playing a bridging role between L-selectin and LFA-1 in this setting.

The expression of $\alpha^4\beta_7$ integrin and L-selectin on lymphocytes varies according to their state of differentiation and activation. Many activated T cells have high levels of $\alpha^4\beta_7$ and are L-selectin-negative — especially those that have originated from the gut [25,27,28]. Importantly, such cells can tether to, and roll on, endothelia *in vivo* without a need for L-selectin. Thus α^4 integrins are capable of supplanting the role of the selectins, but the degree to which they do so may depend on the relative levels of expression of selectins and their ligands compared to the level α^4 integrins and their ligands. The ability of $\alpha^4\beta_7$ integrins to mediate selectin-independent interactions may be especially important for the traffic of activated T cells. The $\alpha^4\beta_1$ integrin may behave in a similar way at other sites of inflammation, where its ligand VCAM-1 is expressed. However, selectins have also been strongly implicated in the adhesion cascade that binds memory T cells to non-mucosal endothelium; E-selectin in inflamed skin binds to ligand(s) expressed on a subset of memory T cells [29,30], and P-selectin operates in a similar manner for an as yet uncharacterized subset of memory T cells [21]. Memory T cells are particularly important for immune surveillance of peripheral tissues and inflammatory lesions [15].

A summary of the probable roles of selectins, α^4 integrins and β_2 integrins/CD31 in the tethering, rolling, and diapedesis (passage through endothelia) of different populations of T cells is shown in Figure 1. This figure, and the papers discussed in this review, emphasize the importance of the differentiation state of a cell in determining its adhesive behavior. Thus, the relative involvement of the selectins and α^4 integrins in cell homing may depend on the properties of both the endothelial cell and the lymphocyte, depending on the homing pathway.

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